abcam

## Product datasheet

# Anti-beta III Tubulin antibody [2G10] (HRP) ab196638

KO VALIDATED

4 Images

### Overview

| | |
|---|---|
| **Product name** | Anti-beta III Tubulin antibody [2G10] (HRP) |
| **Description** | Mouse monoclonal [2G10] to beta III Tubulin (HRP) |
| **Host species** | Mouse |
| **Conjugation** | HRP |
| **Tested applications** | **Suitable for:** IHC-P, WB |
| **Species reactivity** | **Reacts with:** Mouse, Rat, Human<br>**Predicted to work with:** Cow, Common marmoset |
| **Immunogen** | Tissue, cells or virus corresponding to Rat beta III Tubulin. |
| **Positive control** | WB: Human, mouse and rat brain tissue lysates. IHC: Human cerebellum tissue. |

### Properties

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|---|---|
| **Form** | Liquid |
| **Storage instructions** | Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Stable for 12 months at -20°C. Store In the Dark. |
| **Storage buffer** | pH: 7.40<br>Preservative: 0.1% Proclin<br>Constituents: 30% Glycerol, 1% BSA, PBS |
| **Purity** | Affinity purified |
| **Clonality** | Monoclonal |
| **Clone number** | 2G10 |
| **Isotype** | IgG2a |

### Applications

Our Abpromise guarantee covers the use of **ab196638** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

| Application | Abreviews | Notes |
|---|---|---|
| IHC-P | | Use a concentration of 100 - 500 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. |
| WB | | 1/5000. Detects a band of approximately 50 kDa (predicted molecular weight: 50 kDa). |

## Target

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|---|---|
| **Function** | Tubulin is the major constituent of microtubules. It binds two moles of GTP, one at an exchangeable site on the beta chain and one at a non-exchangeable site on the alpha-chain. TUBB3 plays a critical role in proper axon guidance and mantainance. |
| **Tissue specificity** | Expression is primarily restricted to central and peripheral nervous system. |
| **Involvement in disease** | Defects in TUBB3 are the cause of congenital fibrosis of extraocular muscles type 3A (CFEOM3A) [MIM:600638]. A congenital ocular motility disorder marked by restrictive ophthalmoplegia affecting extraocular muscles innervated by the oculomotor and/or trochlear nerves. It is clinically characterized by anchoring of the eyes in downward gaze, ptosis, and backward tilt of the head. Congenital fibrosis of extraocular muscles type 3 presents as a non-progressive, autosomal dominant disorder with variable expression. Patients may be bilaterally or unilaterally affected, and their oculo-motility defects range from complete ophthalmoplegia (with the eyes fixed in a hypo- and exotropic position), to mild asymptomatic restrictions of ocular movement. Ptosis, refractive error, amblyopia, and compensatory head positions are associated with the more severe forms of the disorder. In some cases the ocular phenotype is accompanied by additional features including developmental delay, corpus callosum agenesis, basal ganglia dysmorphism, facial weakness, polyneuropathy. |
| **Sequence similarities** | Belongs to the tubulin family. |
| **Domain** | The highly acidic C-terminal region may bind cations such as calcium. |
| **Post-translational modifications** | Some glutamate residues at the C-terminus are polyglutamylated. This modification occurs exclusively on glutamate residues and results in polyglutamate chains on the gamma-carboxyl group. Also monoglycylated but not polyglycylated due to the absence of functional TTLL10 in human. Monoglycylation is mainly limited to tubulin incorporated into axonemes (cilia and flagella) whereas glutamylation is prevalent in neuronal cells, centrioles, axonemes, and the mitotic spindle. Both modifications can coexist on the same protein on adjacent residues, and lowering glycylation levels increases polyglutamylation, and reciprocally. The precise function of such modifications is still unclear but they regulate the assembly and dynamics of axonemal microtubules. |
| **Cellular localization** | Cytoplasm > cytoskeleton. |

## Images


Western blot - Anti-beta III Tubulin antibody [2G10] (HRP) (ab196638)

**All lanes :** Anti-beta III Tubulin antibody [2G10] (HRP) (ab196638) at 1/5000 dilution

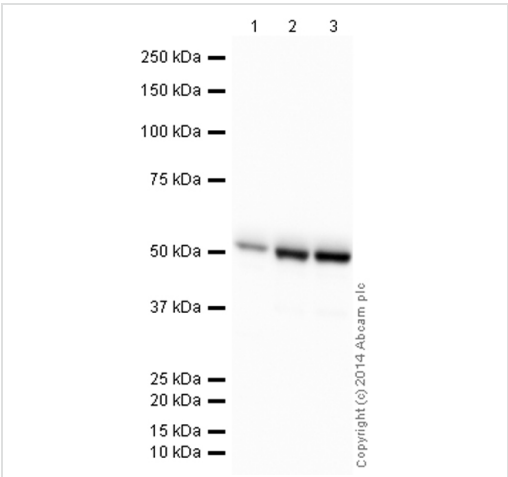
**Lane 1 :** Wild-type HAP1 whole cell lysate
**Lane 2 :** TUBB3 (beta III Tubulin) knockout HAP1 whole cell lysate

Lysates/proteins at 20 µg per lane.

**Predicted band size:** 50 kDa

**Exposure time:** 2 minutes

ab196638 was shown to specifically react with beta III Tubulin in wild-type HAP1 cells as signal was lost in TUBB3 (beta III Tubulin) knockout cells. Wild-type and TUBB3 (beta III Tubulin) knockout samples were subjected to SDS-PAGE. Ab196638 and ab184095 (Mouse monoclonal [mAbcam 9484] to GAPDH - Loading Control (Alexa Fluor® 680) loading control) were incubated overnight at 4°C at 1/5000 dilution and 1/1000 dilution respectively. The loading control was imaged using the Licor Odyssey CLx prior to blots being developed with ECL technique.




Western blot - Anti-beta III Tubulin antibody [2G10] (HRP) (ab196638)

**All lanes :** Anti-beta III Tubulin antibody [2G10] (HRP) (ab196638) at 1/5000 dilution

**Lane 1 :** Brain (Human) Tissue Lysate - adult normal tissue
**Lane 2 :** Brain (Mouse) Tissue Lysate
**Lane 3 :** Brain (Rat) Tissue Lysate

Lysates/proteins at 10 µg per lane.

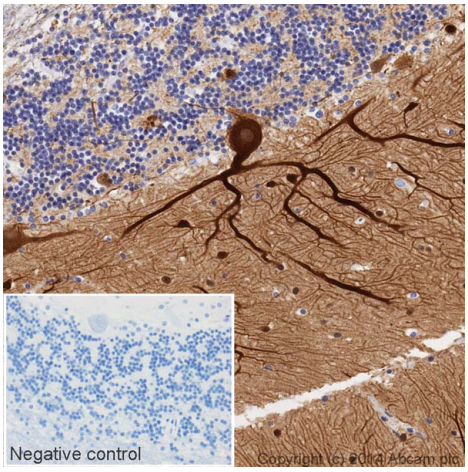
Developed using the ECL technique.

Performed under reducing conditions.

**Predicted band size:** 50 kDa
**Observed band size:** 50 kDa

**Exposure time:** 2 seconds

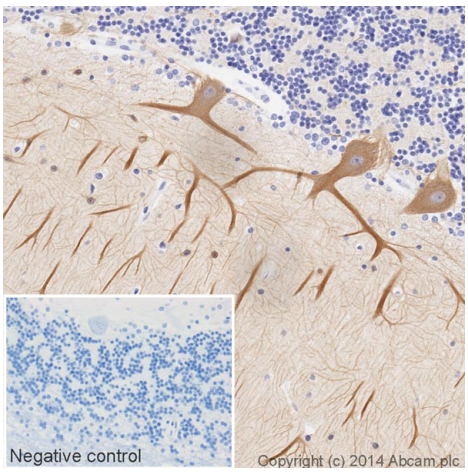
This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 2% Bovine Serum Albumin before being incubated with ab196638 overnight at 4°C. Antibody binding was visualised using ECL development solution ab133406.




Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta III Tubulin antibody [2G10] (HRP) (ab196638)

IHC image of beta III Tubulin staining in a section of formalin-fixed paraffin-embedded normal human cerebellum tissue, performed on a Leica BOND. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab196638 at 1/100 dilution, for 15 mins at room temperature. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset negative control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

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